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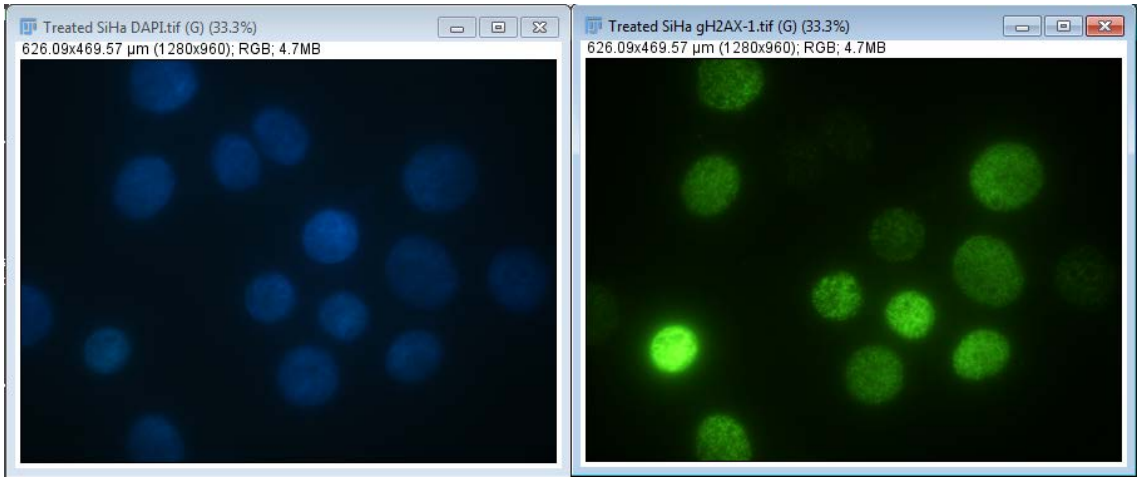
MICROSCOPY NEW ZEALAND INC. CONFERENCE WORKSHOP 2017

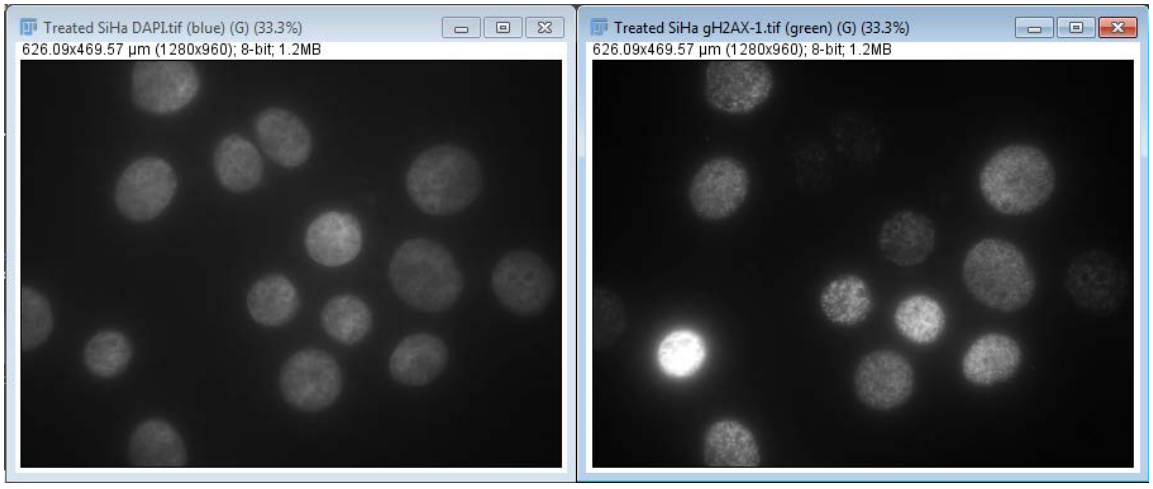
Measuring area and intensity of fluorescent objects with Fiji 31 January 2017

Example 1

1. Open the images called **Treated SiHa DAPI.tif** and **Treated SiHa gH2AX-1.tif**.
2. The image of the nuclei will be used to create a mask for the green image.
3. Calibrate the images using the **20x** image of the micrometer slide.

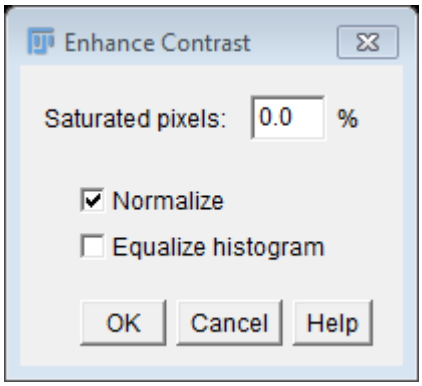


4. Create grayscale versions of both images (***Image – Color –Split channels***). Discard the other channels.

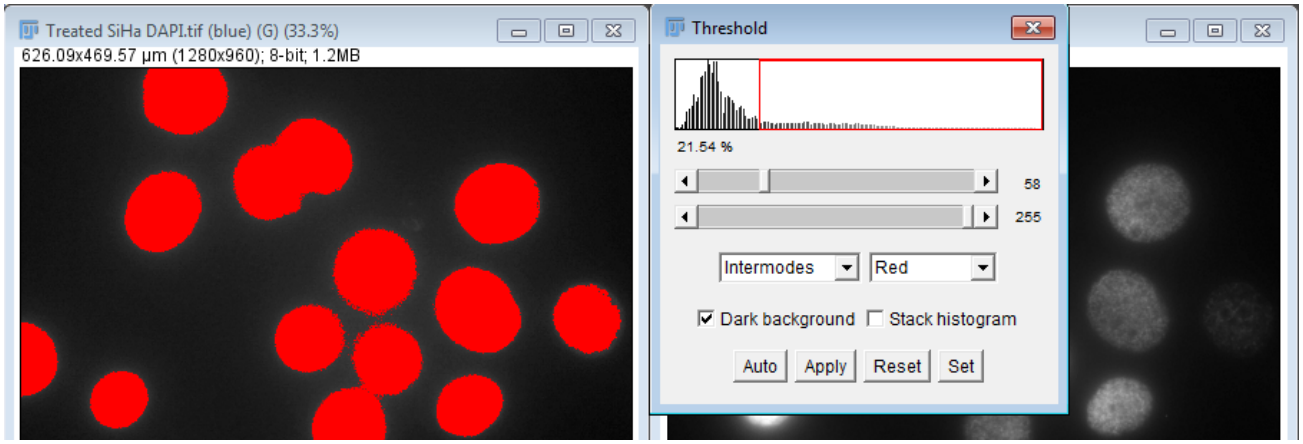


5. Select the **DAPI Blue** image and go to **Process – Enhance Contrast**;

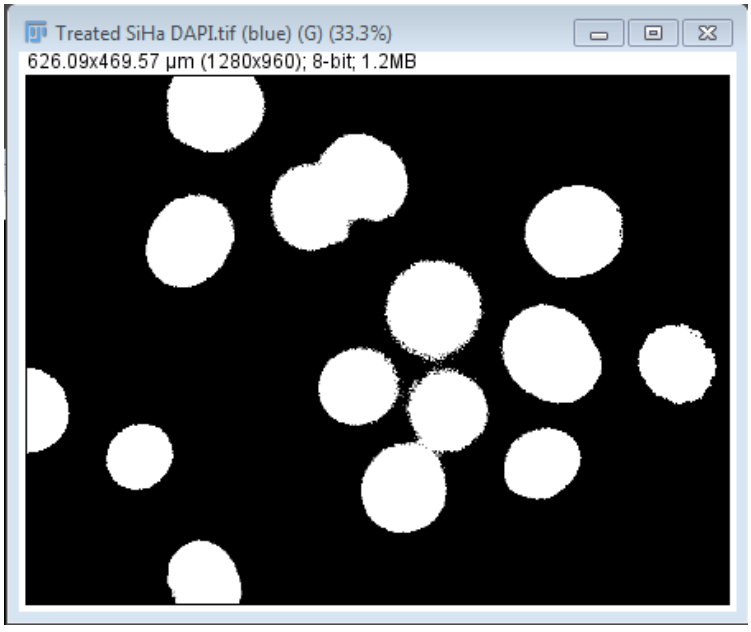
6. Try out the settings until the nuclei look well defined but not oversaturated or too joined together.



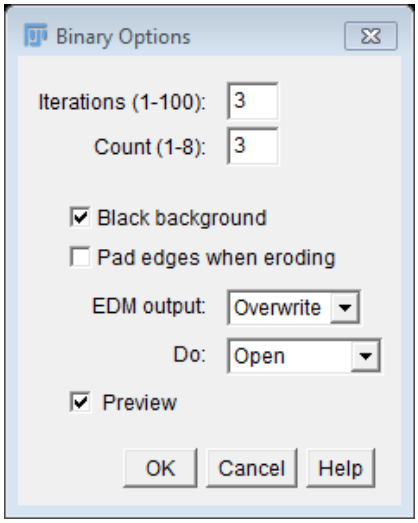
7. Go to **Image – Adjust Threshold** and choose a method that selects the nuclei well;



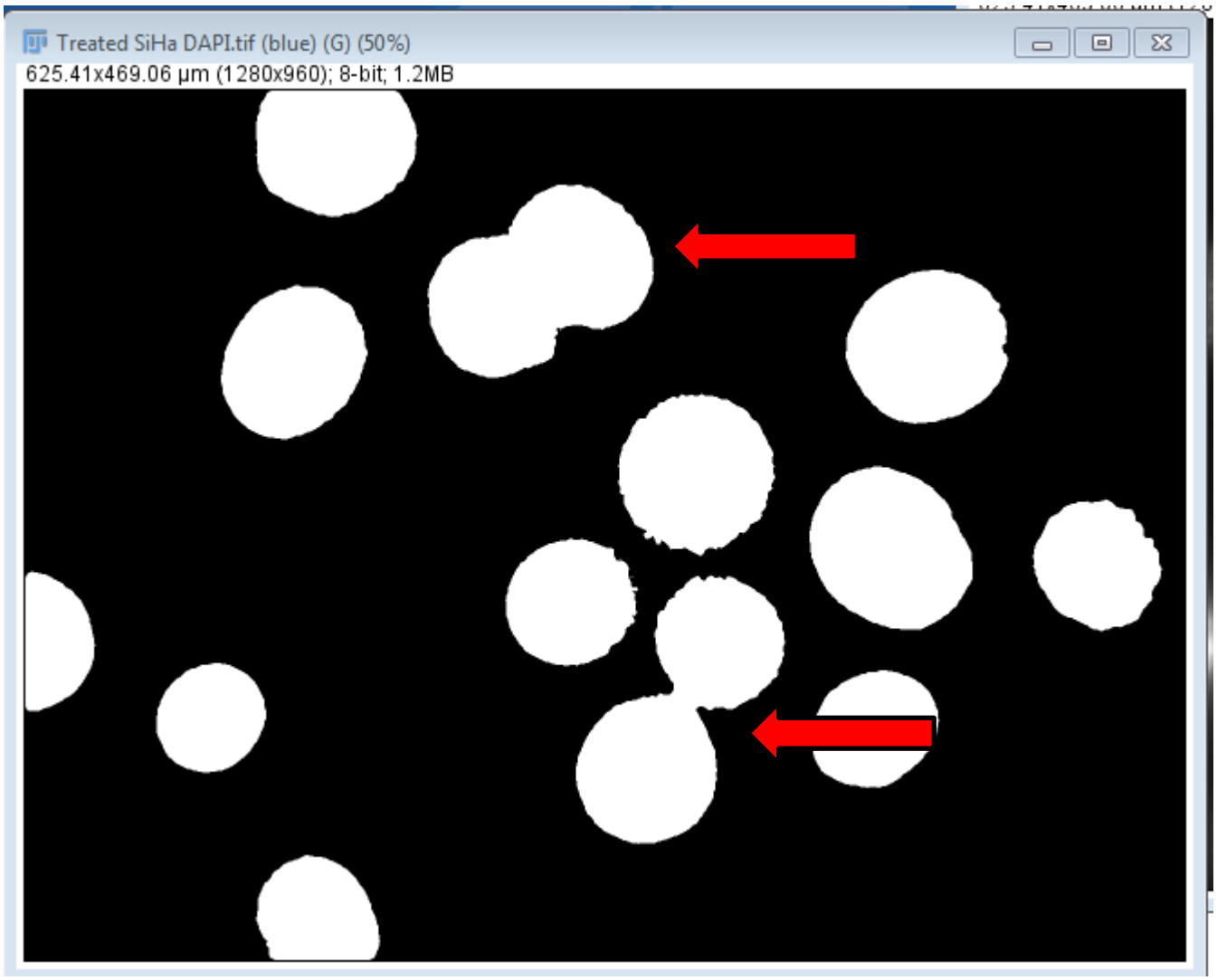
8. Click **Apply** to make the nuclei image binary.

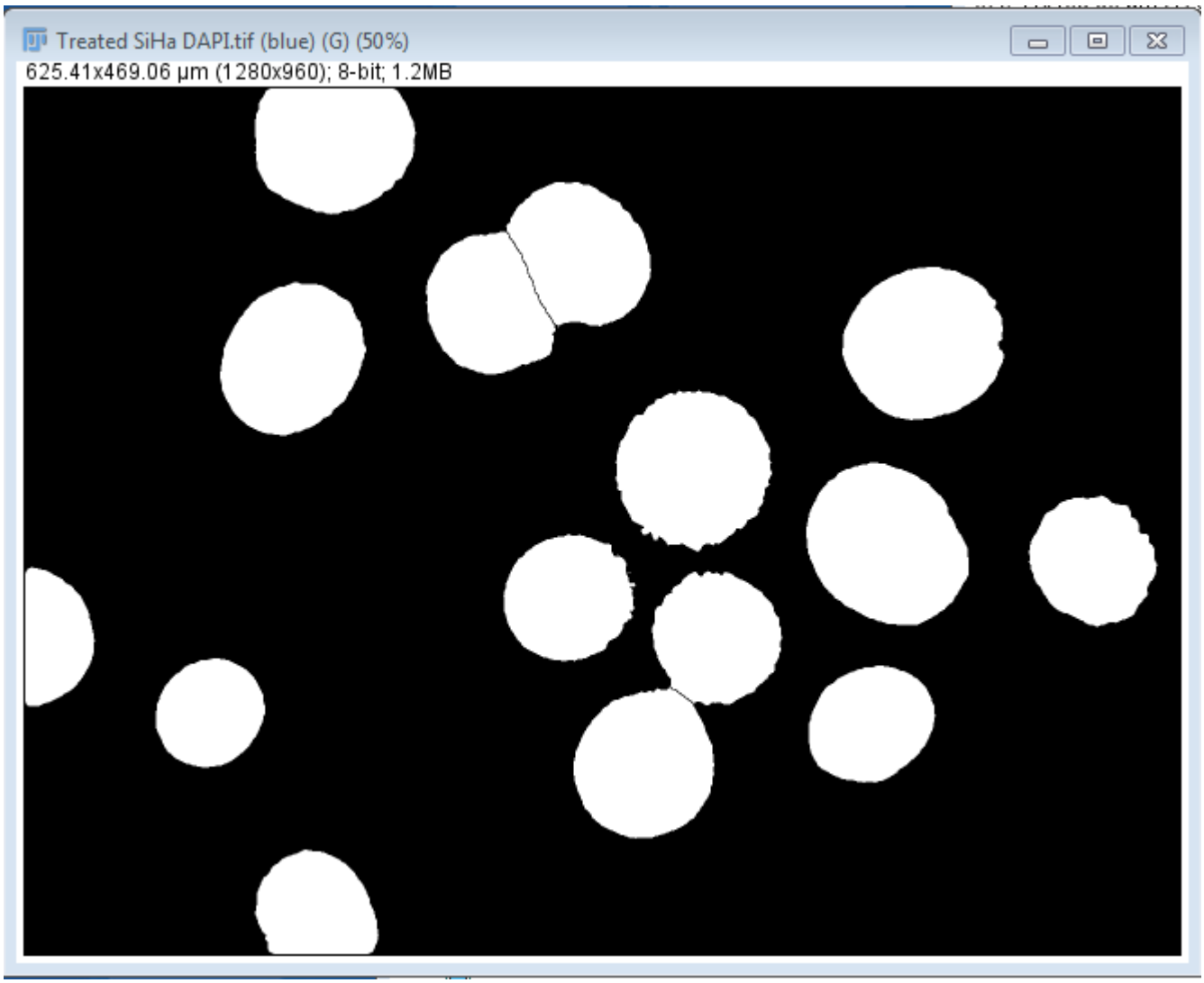


9. We need to remove some small objects that aren't attached to the nuclei. Go to **Process – Binary Options** and try out the different operators and options. Make sure that **Black background** is selected. You need one that smooths the outlines a bit and removes small particles but doesn't take away or add too much. Click **OK** when you are satisfied with the results.

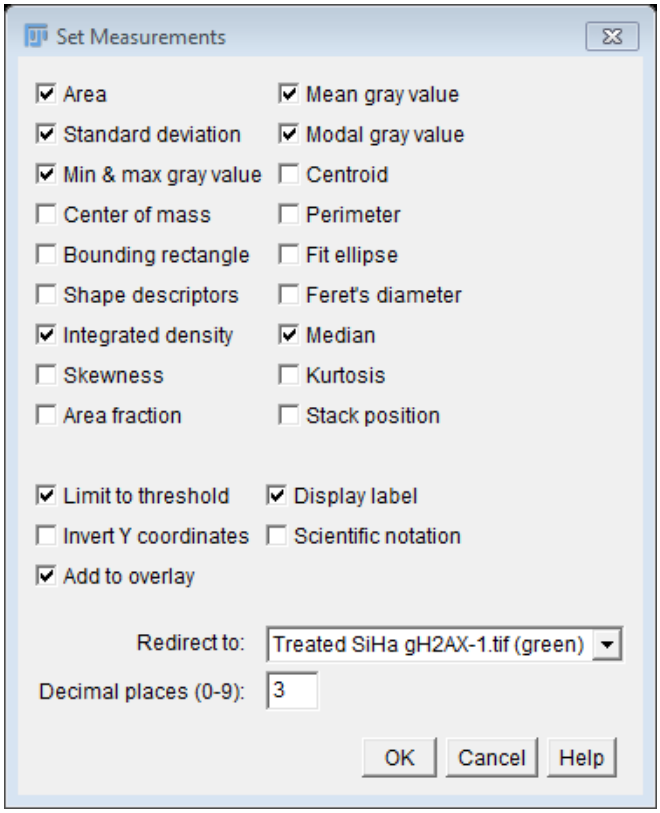


10. Several of the nuclei are joined together so we need to use the **Watershed** operation to separate them.
11. Go to **Process – Binary – Watershed** to separate the joined nuclei;

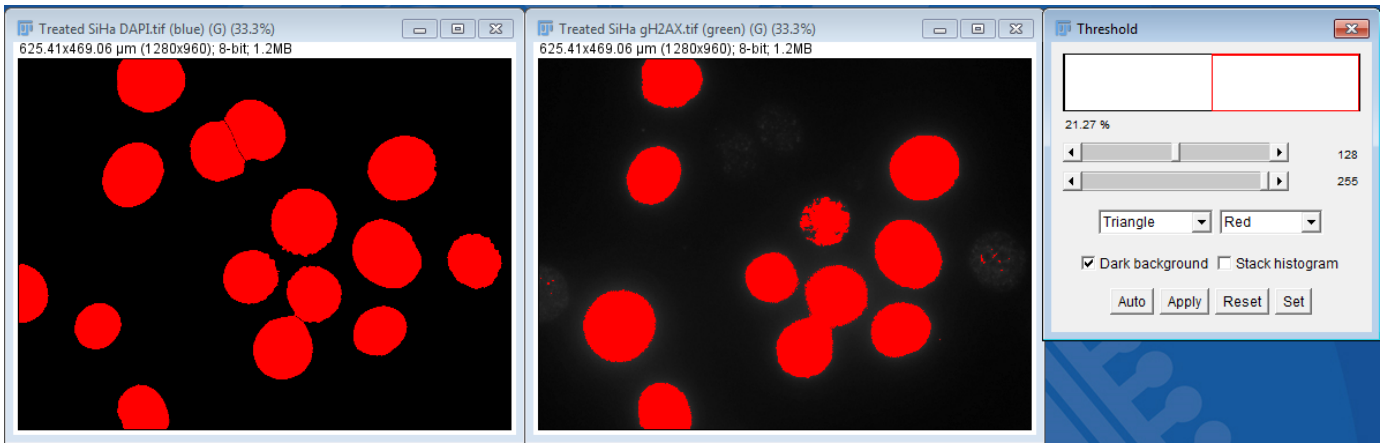




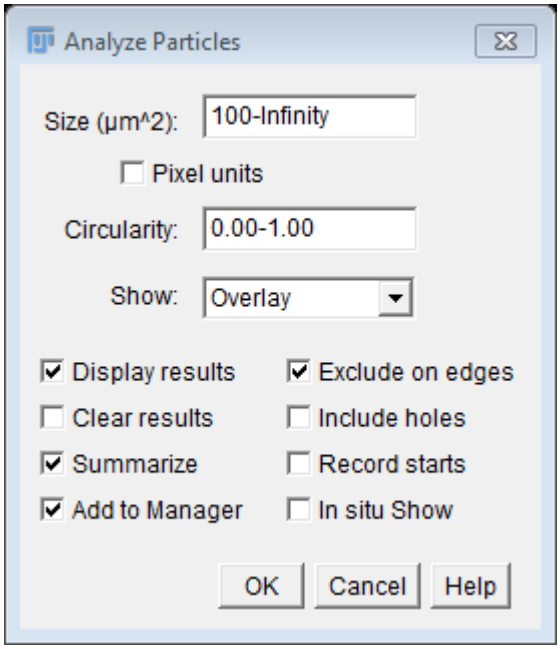
12. Go to **Analyze – Set Measurements** to select the parameters you want to measure. Make sure that you select all of the gray value parameters as well as **Limit to Threshold** and **Display Label**. Select the **Green** image in the **Redirect to**: drop-down box and click **OK**.

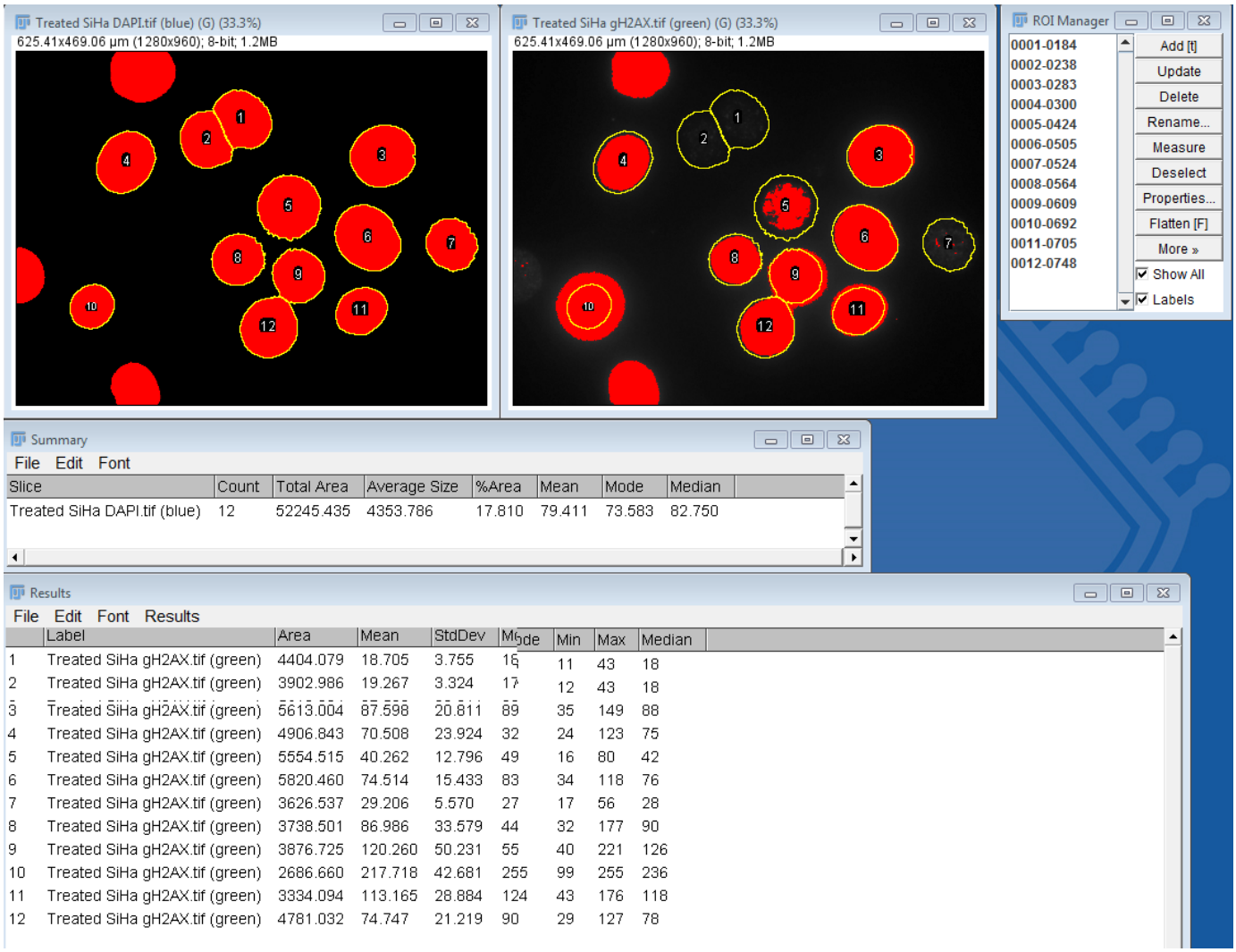


13. Make sure that you have a threshold set for the images (**Image – Adjust – Threshold**);

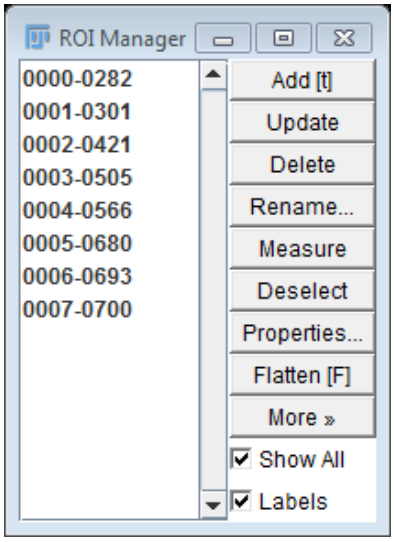


14. Go to **Analyze – Analyze Particles** and select the options you need. You can set a minimum size so that you don't include small objects/debris. You should also **Exclude on edges** in this case.

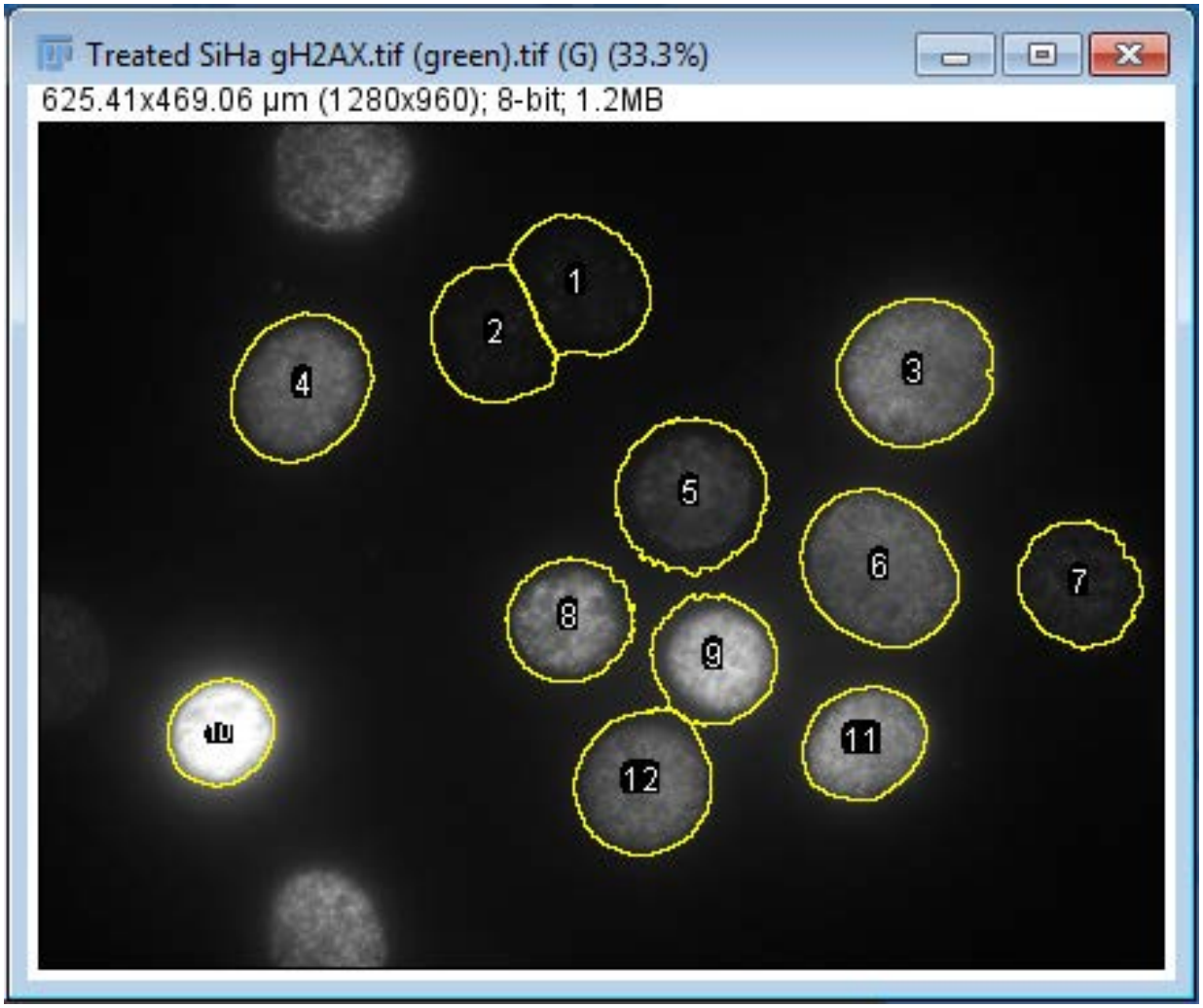

Analyze Particles
Size (µm^2):
100-Infinity
Pixel units
Circularity:
0.00-1.00
Show:
Overlay
Display results
Exclude on edges
Clear results
Include holes
Summarize
Record starts
Add to Manager
In situ Show
OK
Cancel
Help


Treated SiHa DAPI.tif (blue) (G) (33.3%)
625.41x469.06 µm (1280x960); 8-bit; 1.2MB
Treated SiHa gH2AX.tif (green) (G) (33.3%)
625.41x469.06 µm (1280x960); 8-bit; 1.2MB
ROI Manager
0001-0184
0002-0238
0003-0283
0004-0300
0005-0424
0006-0505
0007-0524
0008-0564
0009-0609
0010-0692
0011-0705
0012-0748
Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
Show All
Labels
Summary
File Edit Font
Slice Count Total Area Average Size %Area Mean Mode Median
Treated SiHa DAPI.tif (blue) 12 52245.435 4353.786 17.810 79.411 73.583 82.750
Results
File Edit Font Results
Label Area Mean StdDev Mode Min Max Median
1 Treated SiHa gH2AX.tif (green) 4404.079 18.705 3.755 16 11 43 18
2 Treated SiHa gH2AX.tif (green) 3902.986 19.267 3.324 17 12 43 18
3 Treated SiHa gH2AX.tif (green) 5613.004 87.598 20.811 89 35 149 88
4 Treated SiHa gH2AX.tif (green) 4906.843 70.508 23.924 32 24 123 75
5 Treated SiHa gH2AX.tif (green) 5554.515 40.262 12.796 49 16 80 42
6 Treated SiHa gH2AX.tif (green) 5820.460 74.514 15.433 83 34 118 76
7 Treated SiHa gH2AX.tif (green) 3626.537 29.206 5.570 27 17 56 28
8 Treated SiHa gH2AX.tif (green) 3738.501 86.986 33.579 44 32 177 90
9 Treated SiHa gH2AX.tif (green) 3876.725 120.260 50.231 55 40 221 126
10 Treated SiHa gH2AX.tif (green) 2686.660 217.718 42.681 255 99 255 236
11 Treated SiHa gH2AX.tif (green) 3334.094 113.165 28.884 124 43 176 118
12 Treated SiHa gH2AX.tif (green) 4781.032 74.747 21.219 90 29 127 78

15. If you want to save the **Overlays** as **Regions of Interest** – load them into the **ROI Manager;** (**Image – Overlay – To ROI Manager**). Then go to **More – Save** to save the ROI set.

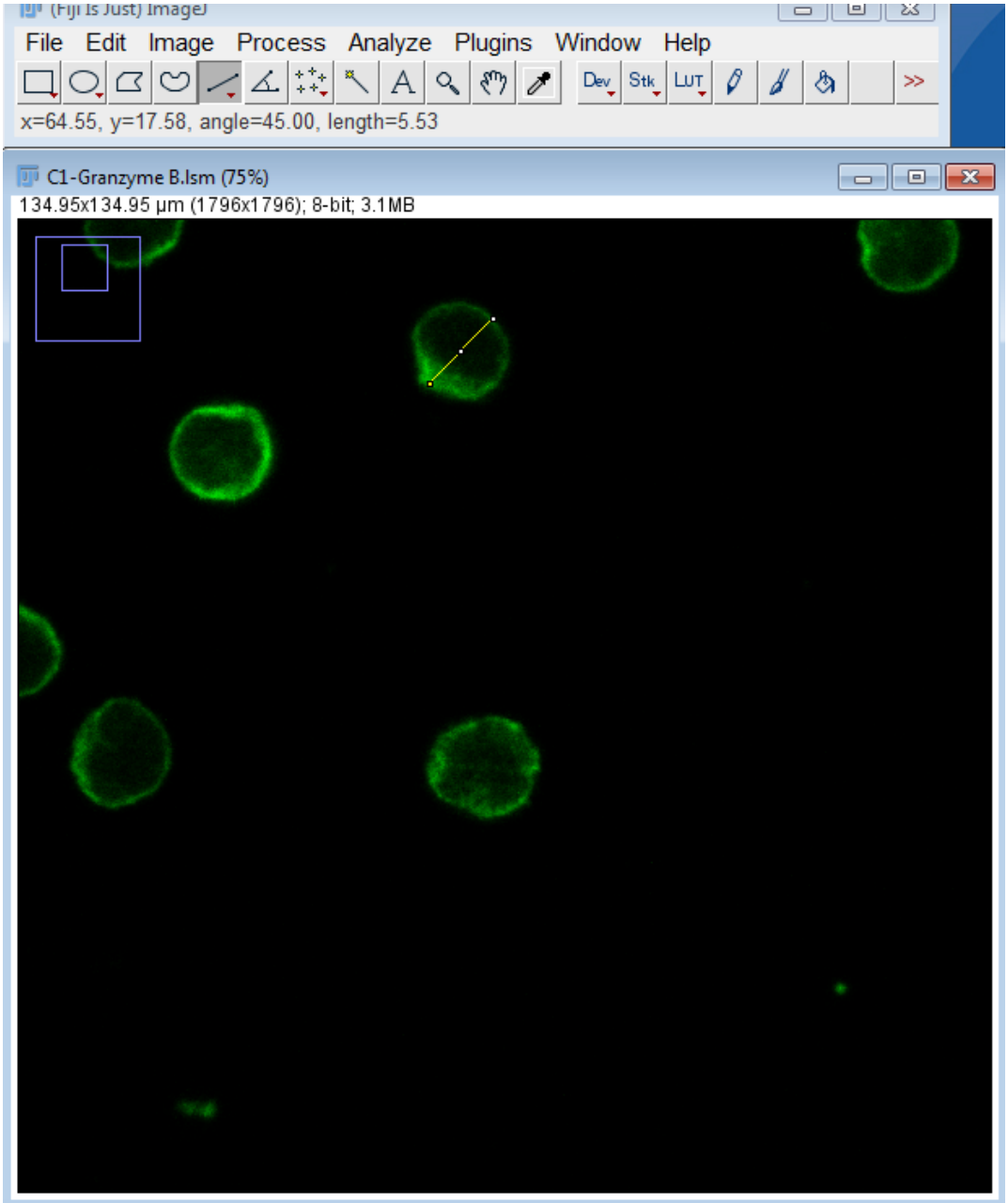


16. If you show the overlay/ROIs on the images (**Show All)** and then save them as TIFF, then the overlay will be saved with the images but not drawn into it. The **Results** and **Summary** files can be saved and opened in Excel or any other software that supports tab-delimited or comma-separated text for further analysis.

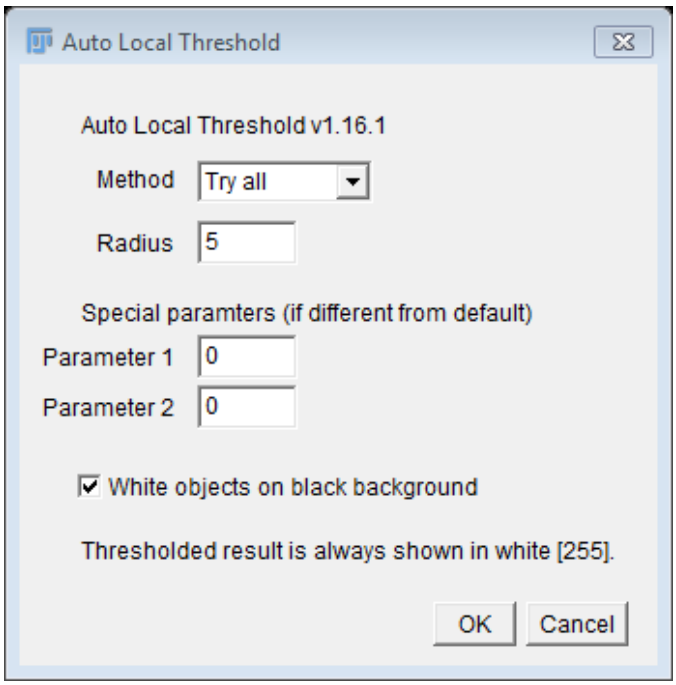


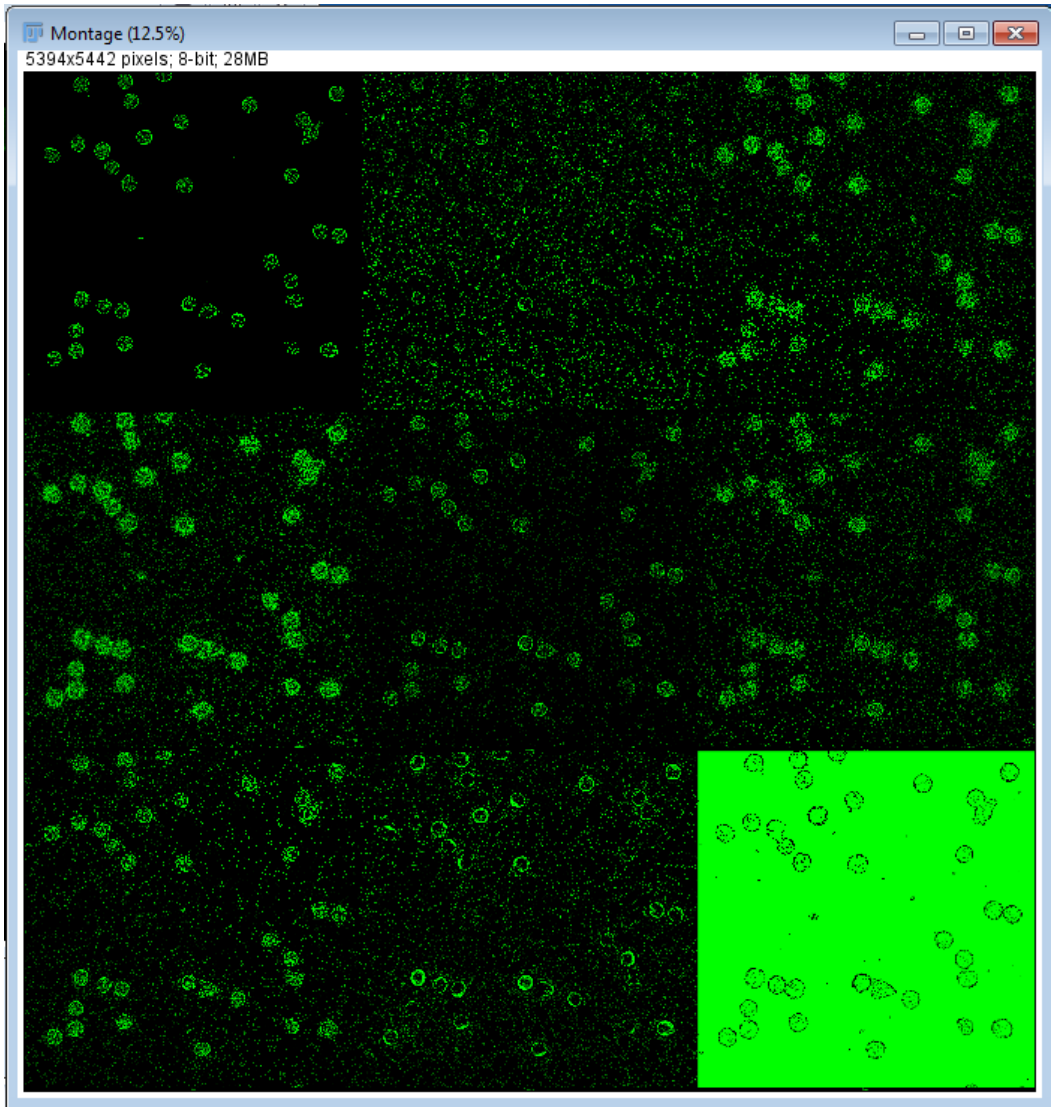
Example 2

1. Open the data set called **Granzyme B.lsm and split it into the 3 components (green, red and gray).**
2. Measure the diameter of the cells on the “green” image by using the **Line tool** (the value is displayed in the **Status bar).** This image will be used for the initial segmentation.

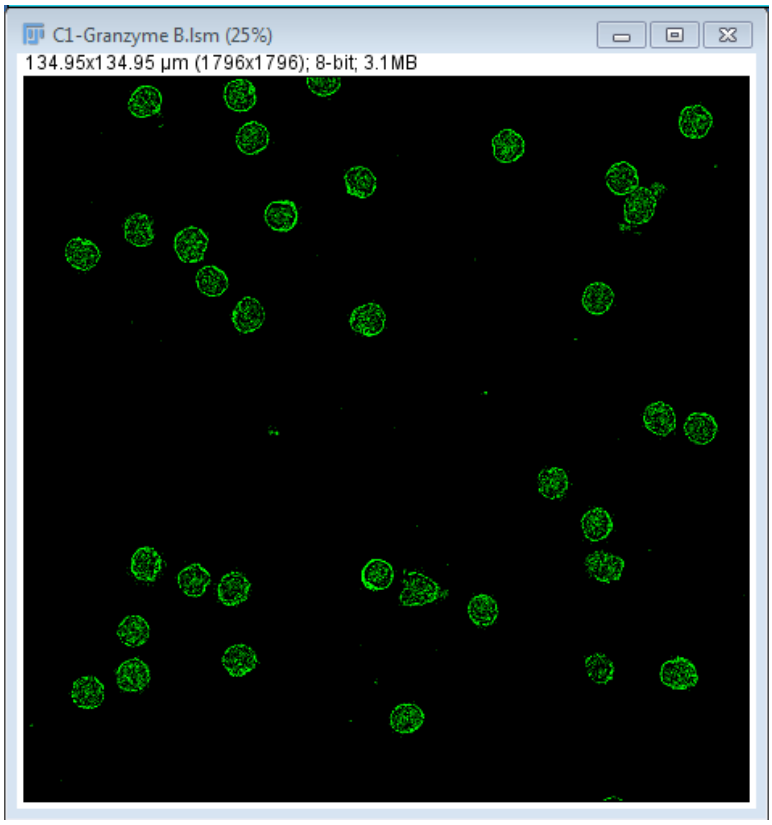


3. Go to **Image-Adjust – Auto Local Threshold** – enter a value for the **Radius** based on your measurement of the cell diameter, e.g. 5 and select **Try all** for the **Method** to see which one will work best.

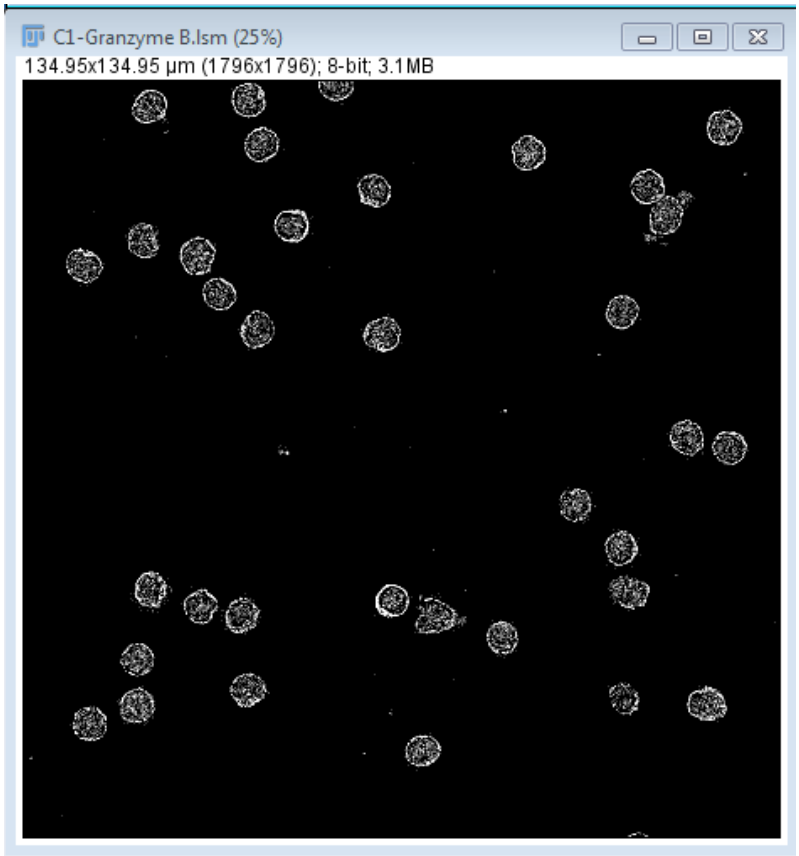

Auto Local Threshold
Auto Local Threshold v1.16.1
Method
Try all
Radius
5
Special paramters (if different from default)
Parameter 1
0
Parameter 2
0
White objects on black background
Thresholded result is always shown in white [255].
OK
Cancel


Montage (12.5%)
5394x5442 pixels; 8-bit; 28MB

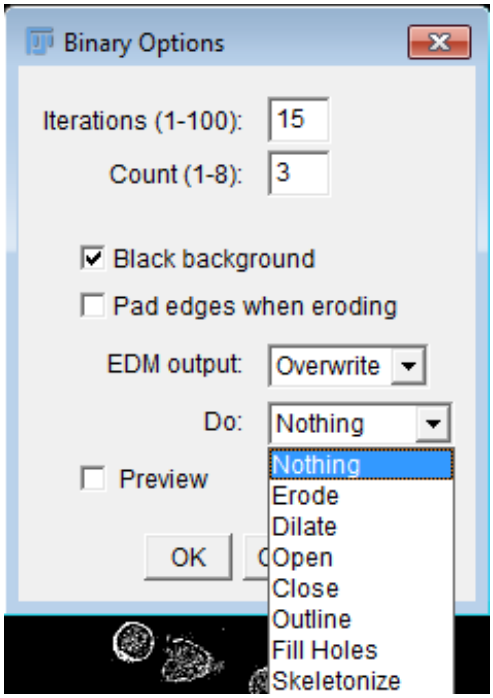
4. Choose the first method = **Bernsen** as it seems the best and run that particular threshold.



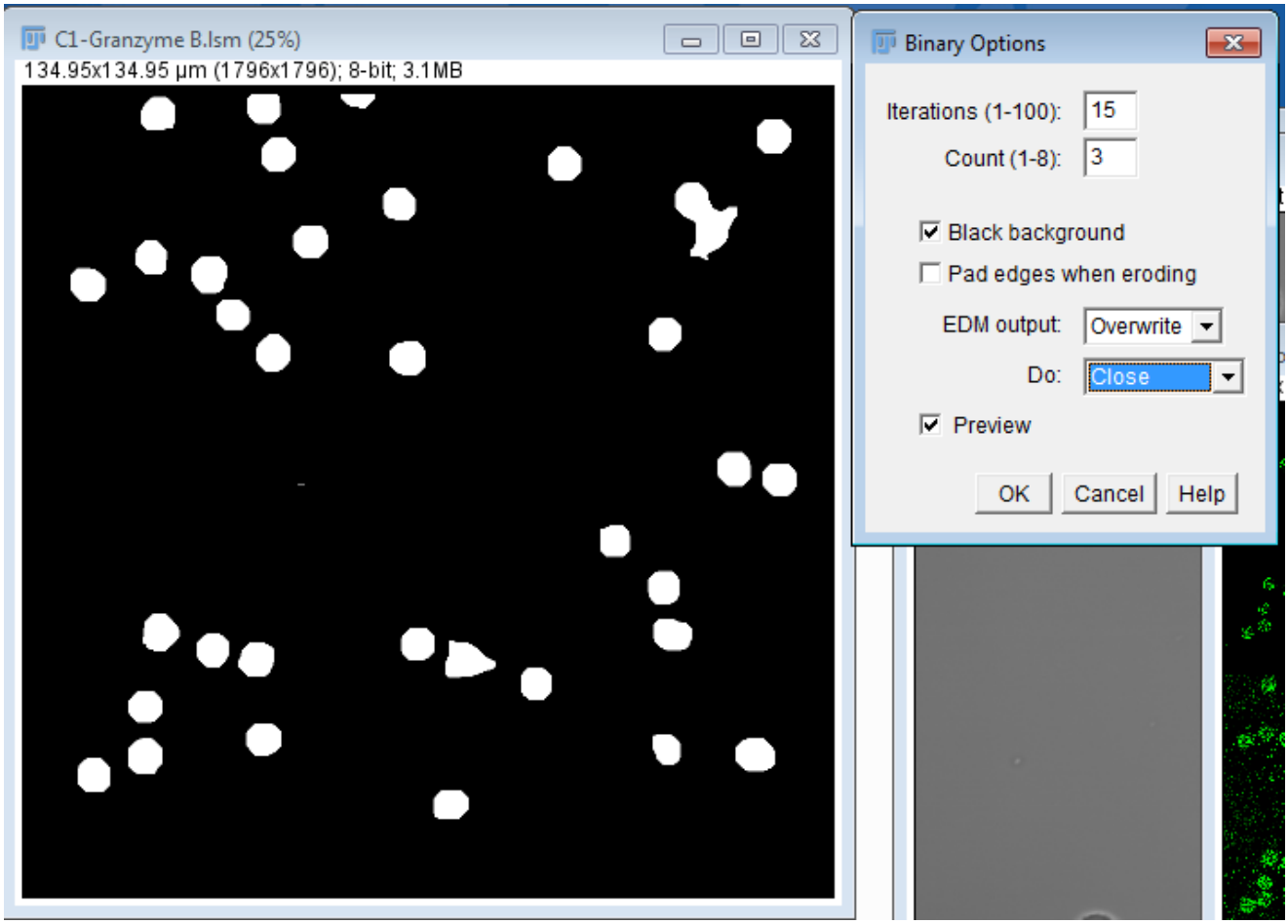
5. Convert the image to binary by going to **Process – Make Binary**;



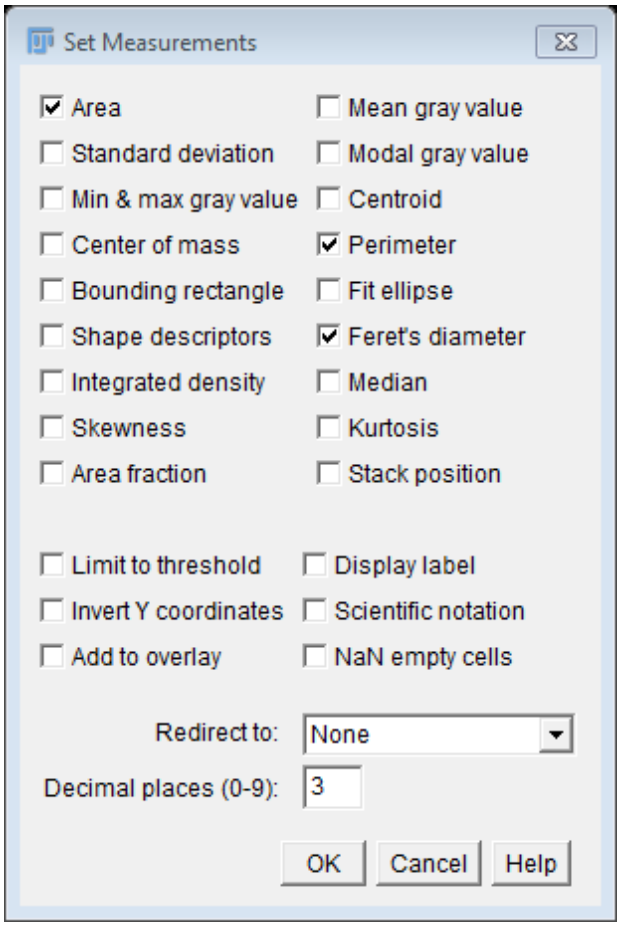
6. Now go to **Process – Binary – Binary Options** and try out the options from the drop-down box varying the number of **Iterations** and **Count values**. The aim is to close and fill the circles.



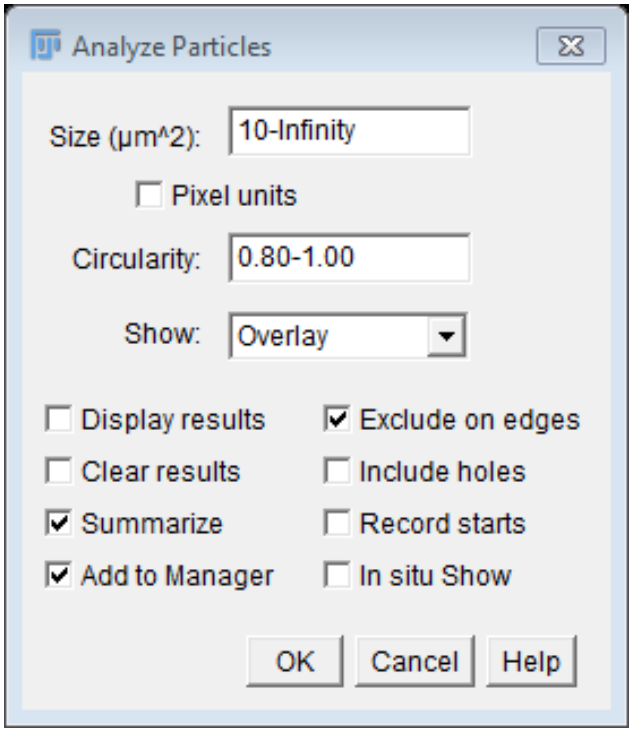
7. The values below work well;

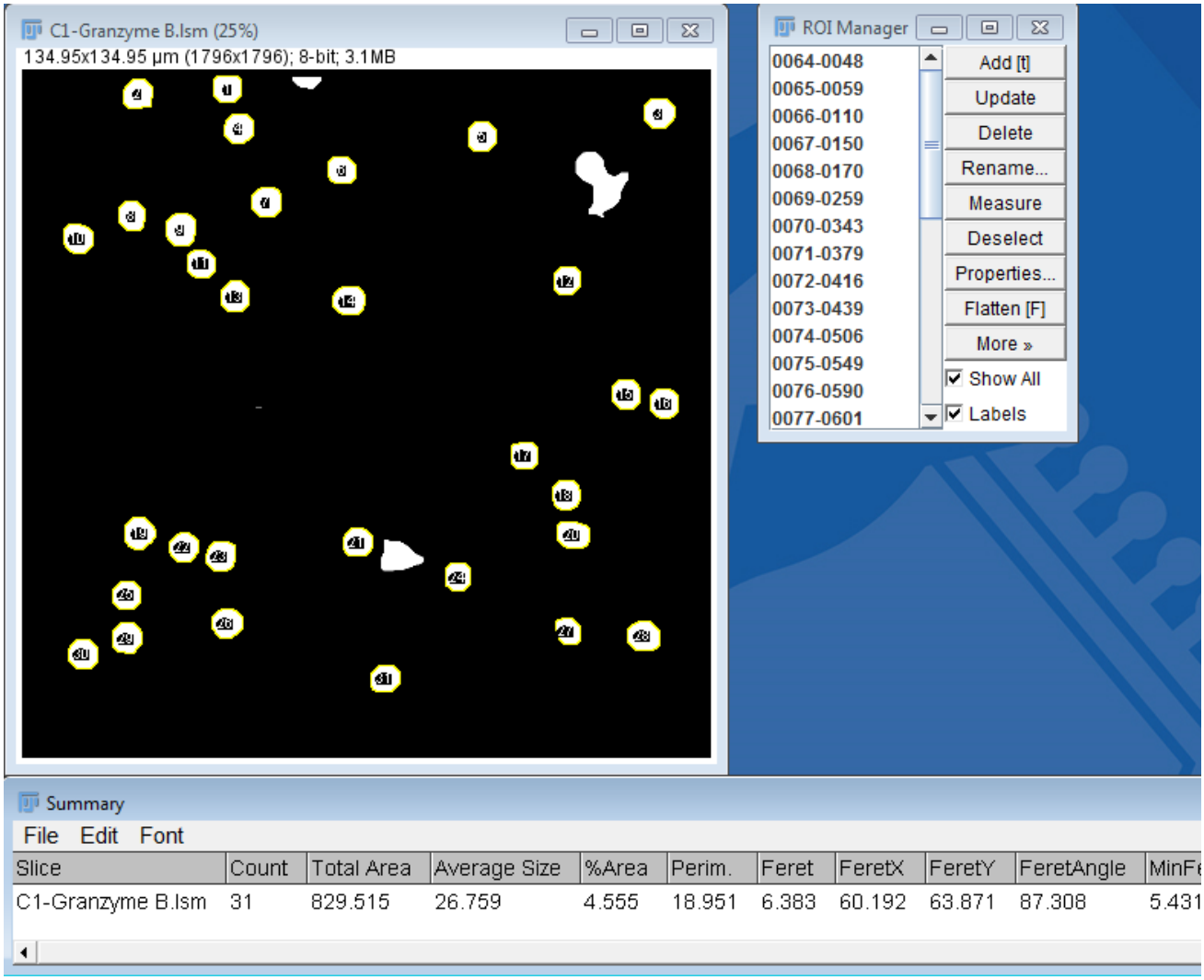


8. Use the following options to create the ROIs for the measurements;

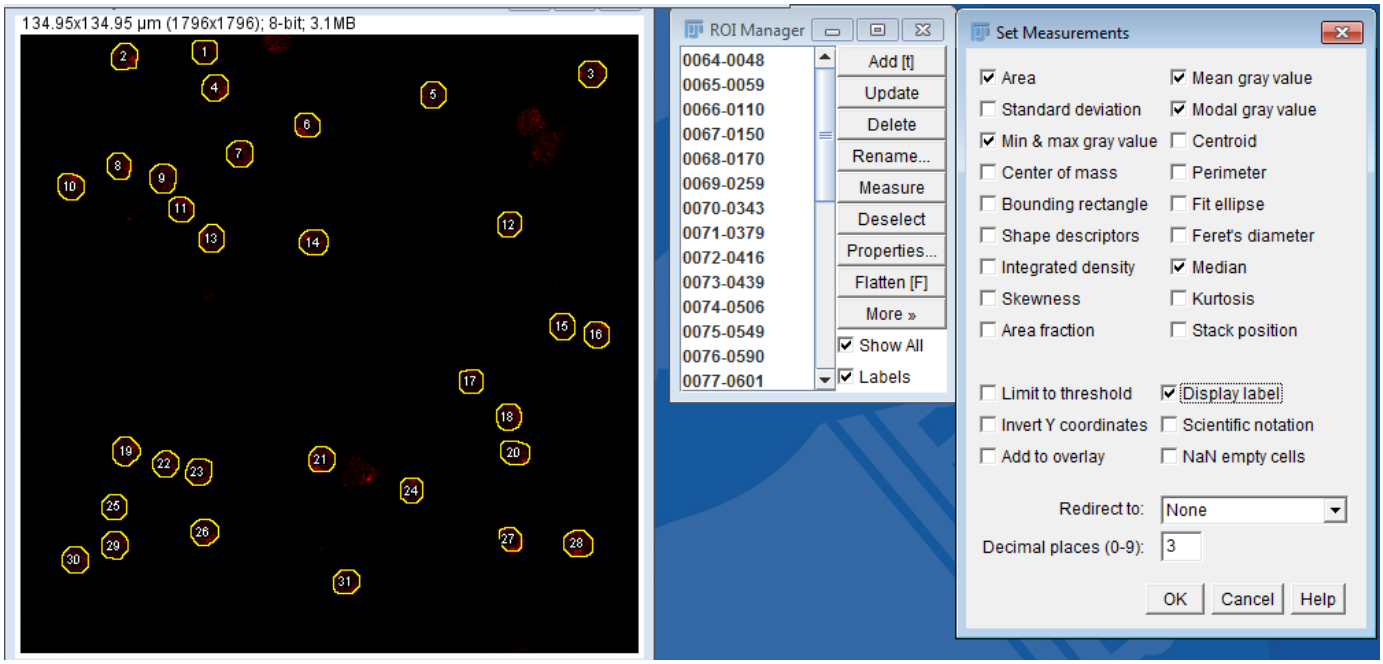


9. Then go to **Analyze – Analyze Particles**. Limiting the analysis to objects that are close to being circular helps to avoid measuring abnormal cells (0.8-1.0).





10. Select the “red” image. Select **Show All** in the ROI Manager to display the outlines. Then go to **Analyze – Set Measurements** and select all of the grayscale value parameters (intensity).



11. Click **Measure** in the **ROI Manager.**

Results

File Edit Font Results

	Label	Area	Mean	Mode	Min	Max	Median
1	C3-Granzyme B.lsm:0064-0048	25.221	30.125	12	1	184	25
2	C3-Granzyme B.lsm:0065-0059	27.519	37.331	13	1	252	27
3	C3-Granzyme B.lsm:0066-0110	28.823	42.953	17	1	227	36
4	C3-Granzyme B.lsm:0067-0150	27.219	38.359	13	1	240	30
5	C3-Granzyme B.lsm:0068-0170	26.926	41.125	25	1	173	37
6	C3-Granzyme B.lsm:0069-0259	25.040	38.758	23	1	255	30
7	C3-Granzyme B.lsm:0070-0343	27.564	46.437	34	1	229	40
8	C3-Granzyme B.lsm:0071-0379	24.967	33.337	13	1	207	26
9	C3-Granzyme B.lsm:0072-0416	31.132	33.896	22	1	207	29
10	C3-Granzyme B.lsm:0073-0439	27.360	42.201	24	1	209	35
11	C3-Granzyme B.lsm:0074-0506	24.871	38.701	14	1	224	30
12	C3-Granzyme B.lsm:0075-0549	25.339	33.807	24	1	221	28
13	C3-Granzyme B.lsm:0076-0590	28.461	42.569	18	1	239	34
14	C3-Granzyme B.lsm:0077-0601	29.195	40.804	22	1	218	34
15	C3-Granzyme B.lsm:0078-0848	27.214	29.527	18	1	204	26
16	C3-Granzyme B.lsm:0079-0871	26.322	37.157	15	1	255	29
17	C3-Granzyme B.lsm:0080-1007	23.645	32.259	13	1	197	28
18	C3-Granzyme B.lsm:0081-1111	25.526	40.460	25	1	218	34
19	C3-Granzyme B.lsm:0082-1210	29.800	39.348	20	1	227	34
20	C3-Granzyme B.lsm:0083-1215	28.501	31.640	1	1	255	24
21	C3-Granzyme B.lsm:0084-1235	26.028	60.479	45	1	234	53
22	C3-Granzyme B.lsm:0085-1249	25.881	34.109	20	1	189	26
23	C3-Granzyme B.lsm:0086-1270	28.185	32.630	15	1	169	27
24	C3-Granzyme B.lsm:0087-1324	23.916	37.960	23	1	203	31
25	C3-Granzyme B.lsm:0088-1374	25.091	24.410	14	1	113	21
26	C3-Granzyme B.lsm:0089-1447	27.242	37.887	17	1	238	30
27	C3-Granzyme B.lsm:0090-1469	20.292	47.812	14	1	255	36
28	C3-Granzyme B.lsm:0091-1480	31.076	58.957	43	1	255	47
29	C3-Granzyme B.lsm:0092-1484	28.845	30.389	12	1	160	26
30	C3-Granzyme B.lsm:0093-1527	27.089	35.599	21	1	231	30
31	C3-Granzyme B.lsm:0094-1592	25.226	40.749	22	1	186	35